

Predominance of fabG1 -15C>T in Mycobacterium tuberculosis isolates in Slovenia (2020 – 2024): A Whole genome sequencing study of drug resistance and transmission

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Abstract

Tuberculosis (TB) is well-managed infectious disease in overall Slovenian population. However, the disease still represents a challenge in several marginal subpopulations. This study analysed 342 *Mycobacterium tuberculosis* (MT) from patients with newly diagnosed TB patients between 2020 and 2024 using phenotypic drug susceptibility testing (pDST) and whole-genome sequencing (WGS). Drug resistance was rare, detected in only 7.60% (26/342) of cases, with isoniazid resistance being the most prevalent (18/26; 69.23%). WGS showed high agreement with pDST, revealing only three discrepancies. The most frequent mutations were *fabG1* -15C > T (10/26; 38.46%) and *katG* Ser315Thr (6/26; 23.08%). Lineage 4 predominated (326/342; 95.32%), while other lineages appeared only sporadically, primarily in foreign-born individuals. Recent transmission likely accounted for 19.01% (65/342) of TB cases. The predominance of the *fabG1* -15C > T mutation suggests a population-specific resistance pattern and unique local transmission dynamics, highlighting the importance of genomic surveillance for targeted TB control in specific risk groups.

Introduction

World health organization (WHO) recognized tuberculosis, disease caused by *Mycobacterium tuberculosis* (MT), as a global health problem three decades ago. Tuberculosis (TB) is preventable and mostly curable disease. Despite global efforts, TB is still the leading cause of death from infectious disease. In 2024, 10.7 million people developed TB and 1.23 million died, highlighting the need to shorten the time to effective therapy and expand the access to timely, comprehensive drug-resistance result^{1,2}. Potential global threat represents the spread of drug-resistant TB, especially multi-drug resistant (MDR) TB from high-burden populations and regions. Recently published systematic review found that while global MDR/RR-TB prevalence has significantly declined, Africa continues to bear a disproportionate burden, particularly among retreatment cases, young adults, and HIV-positive patients³.

Slovenia is European country with low incidence of TB. Incidence of TB dropped below 10 in 2009, and it is declining ever since. After 2018, TB incidence rate never exceeded value of 5.0; in 2024, we observed incidence rate to be only 2.3, the lowest so far, followed by slight increase in TB incidence rate (3.7) in 2025⁴. Although tuberculosis is well managed in overall population in Slovenia, the disease still represents a challenge in several marginal subpopulations (e.g. construction workers, residents of social welfare centres etc.)

In the past decades, several DNA typing methods, including IS6110 restriction fragment length polymorphism typing (RFLP), mycobacterial interspersed repeat unit-variable number of tandem repeat typing (MIRU-VNTR) and spoligotyping, have been developed to genotype and study epidemiology of MT strains. Their main disadvantage was insufficient resolution and often time-consuming and technically demanding workflow⁵. On the other hand, whole genome sequencing (WGS) allows analysing sequencing data by comparison to MT H37Rv as a reference genome due to highly conservative nature of MT genome. Nowadays, WGS represent reliable clinical application with high throughput, high

resolution and its simultaneous identification of MT species, drug resistance detection, genotyping and cluster identification^{5–8}. Furthermore, WGS also enables transmission outbreak detection and monitoring of trends in drug resistance⁵. Analysing single nucleotide polymorphisms (SNPs) in MT isolates is frequently used to identify clusters of TB patients that might help to investigate the dynamics of TB transmission⁹. For cluster definition, pairwise distance between MT strains of < 12 SNPs has been set as a threshold and < 5 SNPs for likely recent transmission events¹⁰.

Widespread use of WGS technology without proper standardization may result in data and applications that lack harmonization, comparability and validation. In recent years, there have been several attempts towards standardisation of WGS for detection of epidemiologically linked TB cases and detection of genetic alterations linked with drug resistance. In one study, five different pipelines (one core genome multilocus sequence typing and four single nucleotide polymorphism-based pipelines) were compared. Despite differences in the pipelines, for identification of highly epidemiologically related MT strains the performance was generally similar. Moreover, concordance in ruling out epidemiological links was high between pipelines, which is an important step in international validation of WGS data analysis. Nevertheless, different clustering rates were still observed reflected by different genetic distances observed between pipelines¹¹.

In 2023, WHO published the second catalogue of mutations associated with drug resistance. The main goal of WHO catalogue of mutations is to guide end users in the interpretation of sequencing data¹². WGS combined with WHO catalogue of mutations enables rapid detection of resistance compared to traditional phenotypic susceptibility testing.

The main goals of our study were to (a) retrospectively sequence and analyse WGS data of all MT isolates from patients with newly discovered TB between years 2020 and 2024, to (b) detect genetic alterations linked with antibiotic resistance, (c) to genotype them and to (d) detect possible transmission/epidemiological links between MT isolates included in this study.

Results

Basic characteristics:

Between 2020 and 2024, 344 patients with newly diagnosed tuberculosis and isolated bacilli of *M. tuberculosis* complex were detected. Two of those isolates were identified as *Mycobacterium bovis* subsp. BCG and were therefore excluded from further analysis. The remaining 342 isolates were identified as MT and were subject for further WGS analysis. Basic demographic characteristics (median age at the time of diagnosis and gender) as well as clinical manifestation of disease of TB patients are presented in Table 1. Median age of all TB patients included in this study was 61.0 (range: 1.0–96.0). Majority of included TB patients were males (202/342; 59.06%). MT isolates were mainly detected in pulmonary samples (252/342; 73.39%) indicating pulmonary TB. The remaining (90/342; 26.32%) were isolated from other body parts indicating extrapulmonary TB.

Phenotypic drug susceptibility testing:

The prevalence of phenotypic drug resistance is presented in Table 2. Briefly, the most prevalent was phenotypic resistance to INH, detected in 69.23% (18/26) of all drug-resistant TB cases. The following were resistance to ETH (12/26; 46.15%) and SM (9/26; 34.62%). Phenotypic RMP resistance was detected in 23.08% (6/26). Nine (9/26; 34.62%) MT isolates were phenotypically resistant to one antibiotic, twelve (12/26; 46.15%) MT isolates were poly resistant and the remaining five (5/26; 19.23%) were phenotypically either multi-drug resistant (MDR) or pre-extensively-drug resistant (pre-XDR). Cases of extensively resistant tuberculosis were not observed in our study.

WGS data analysis:

Majority of MTs included were successfully sequenced; for six MT isolates the median coverage was below 30x and were therefore sequenced again. Second WGS gained coverages over 30x.

- Correlation between phenotypic DST and WGS analysis:

Table 3 represents correlation between pDST and WGS analysis for all 26 (26/342; 7.60%) MT drug resistant isolates identified in the frame of our study.

Discrepancy between pDST and WGS was observed in three MT isolates. In one MT isolate with phenotypic PZA resistance, no mutations were found; phenotypic PZA susceptibility testing was repeated several times and it always revealed PZA resistance. In another MT isolate phenotypic resistance to RMP and ETH was revealed and confirmed with *rpoB* Gln436del and *inhA* - 796C > T, respectively. Furthermore, mutation *inhA* - 796C > T might also confer INH resistance; however, that isolate tested INH susceptible. The third MT isolate harboured mutation *gyrA* Val624Leu although it was phenotypically sensitive to fluoroquinolones. After repeated pDST for fluoroquinolones MT isolate remained phenotypically susceptible.

Majority of Beijing MT isolates (3/4; 75%) were resistant to at least five antibiotics or more, one Beijing isolate was resistant to two antibiotics, INH and ETH. In all Beijing cases, resistance was confirmed with WGS. Interestingly enough, mutation *rrs* 517C > T conferring SM resistance was found only in two Beijing isolates. SM resistance in other lineages was linked with mutations in *rpsL* or *gid* genes but not in *rrs* gene.

- Prevalence of mutations linked to drug resistance:

Altogether, 39 mutations linked to drug resistance were discovered in the frame of this study (Table 5). The most prevalent mutation was *fabG1* -15C > T conferring ETH and low-level INH resistance in 10 MT isolates (10/26; 38.46%). The second most prevalent mutation was *katG* Ser315Thr that was found in six MT isolates (6/26; 23.08%) with phenotypic INH resistance. Other mutations linked to INH resistance in genes *katG*, *inhA* and *ahpC* were found in lower frequencies. Mutations linked with phenotypic RMP

resistance were found only in *rpoB* gene and in lower frequencies. Mutations linked with either SM, EMB, PZA, fluoroquinolones (FLQ) and KN were found in low frequencies as presented in Table 5.

- M. tuberculosis lineages:

Majority of MT isolates (326/342; 95.32%) belonged to lineage 4. Lineage 2 was represented in 2.92% (10/342), and lineages 1 and 3 in less than 1%. Among lineage 4, sub lineage 4.1.2.1 (Haarlem) was the most prevalent one and was present in 131 (131/342; 38.30%) patients with newly diagnosed TB. Sub lineage 4.8 (mainly T) was also detected in higher proportion of patients with newly diagnosed TB (80/342; 23.39%). Distribution of lineages is presented in Table 2.

Distribution of lineages among resistant MT isolates is presented in Table 2. Briefly, majority of isolates belonged to lineage 4 (19/26; 73.08%). However, resistant MT isolates characterized as lineage 4 represented only 5.86% (19/342) of all lineage 4 MT isolates. The observed percentages of resistant MT isolates are higher in other lineages: 33.33% (1/3) of resistant MT isolates for lineage 1, 40.00% (4/10) for lineage 2 and 33.33% (1/3) for lineage 3.

- Phylogenetic analysis:

Maximum likelihood trees for all MT isolates included in this study and for MT isolates identified as lineage 4 are presented in Figs. 1 and 2.

By applying a threshold of 12 SNPs, 104 (104/342; 30.41%) MT isolates were clustered into 31 clusters with at least two MT isolates. Among them, 14 clusters had at least tree isolates. The remaining 238 (238/342; 69.59%) isolates were unclassified. The largest cluster comprised of 11 MT isolates. In three of those MT isolates mutation – 15C > T in *fabG1* conferring resistance to INH and ETH emerged.

Using a 5-SNP threshold, 65 (65/342; 19.01%) MT isolates were clustered into 27 clusters with at least two MT isolates. Among them, five clusters had at least tree isolates.

Among MT isolates belonging to lineages 1, 2 or 3, only three MT isolates identified as lineage 2 were clustered together. Among those three, only two were less than 5 SNPs apart indicating close relatedness.

Discussion

This is the first WGS study of clinical MT isolates in Slovenia that includes all newly discovered TB patients between 2020 and 2024. In the investigated period of five years, TB incidence was already below 5.0 which represents current situation in Slovenia. Selecting older period, when TB incidence and resistance were higher, might gave us completely different picture regarding epidemiological situation and resistance prevalence. Nowadays, tuberculosis is well-managed infectious disease in overall Slovenian population. However, the disease still represents a challenge in several marginal subpopulations (such as construction workers, residents of social welfare centres, some ethnic groups) where the largest transmission clusters were observed.

Observed drug resistance is rare in Slovenia, only 7.60% (26/242) of TB patients were resistant to at least one antibiotic. In the studied population, the most prevalent was resistance to INH, detected in 69.20% (18/26) of all patients with drug resistant TB; the resistance was mainly linked with promoter mutation – 15C > T in *fabG1* (10/18, 55.60%), followed by *katG* mutations (8/18, 44.40%). Among them *katG* Ser315Thr was the most prevalent one (6/18; 33.30%). Global meta-analysis¹³ categorized *fabG1* -15C > T alongside *katG* Ser315Thr and *rpoB* Ser531Leu as the definitive canonical triad of mutations responsible for first-line treatment failure. While *katG* mutations are mainly destructive to the activity of catalase peroxidase KatG, promoter mutation *fabG1* -15C > T selectively causes low level resistance to INH and cross-resistance to ETH, allowing MT strains to circulate aggressively in population with low fitness costs¹⁴. According to published literature, *katG* mutations dominate globally, while certain geographic cohorts present high prevalence of the *fabG1* -15C > T promoter mutation due to local clonal transmission^{13,15–18}. A notable finding of this study is the predominance of *fabG1* -15C > T in INH resistant and in all drug-resistant cases suggesting population-specific resistance pattern. The mutation occurred almost exclusively in the absence of concomitant *katG* mutations. We identified only one case of INH resistant MT where both *fabG1* -15C > T and *katG* Ser315Thr were detected. That situation might be attributed to several factors, including successful transmission and expansion of resistant MT clones, potential fitness advantages associated with promoter-mediated INH resistance, and specific local transmission dynamics. Unlike *katG* mutations, *fabG1* promoter mutations preserve KatG function and may impose a lower fitness burden, facilitating persistence and spread within the studied population. Most probably clonal transmission happened decades ago due to selective pressure. To fully understand what was the reason of *fabG1* -15C > T predominance in our population it would be interesting enough to sequence MT isolates, especially the cases with INH resistance, that emerged before 2020.

Among the TB cases with detected *fabG1* -15C > T in our study, five cases were epidemiologically linked: three cases belonged to an outbreak that happened among construction workers and two cases were observed within family members. The other five cases remained unlinked. The identification of epidemiologically linked cases carrying *fabG1* -15C > T suggests that transmission of resistant clones contributes to the observed prevalence of this mutation. Furthermore, the presence of *fabG1* -15C > T mutation in unlinked cases suggest independent emergence or older transmission events or even support both scenarios. Although predominantly observed in lineage 4 MT isolates, detection of *fabG1* -15C > T mutation in lineage 2 MT isolate indicates that this resistance-associated variant can emerge across diverse genetic backgrounds. To support older transmission events, TB cases between 2020 and 2024 were sequenced and consequently transmission chains extending beyond the sampled population were most probably missed.

Cases of MDR and pre-XDR are even rarer in Slovenian population, all together representing 1.46% (5/342) of all observed TB cases in our study. Those cases were mainly detected in MT isolates belonging to lineage 2 (3/5; 60%). This observation is concordant with reports from other European countries, where the vast majority of MDR and/or XDR cases belonged to lineage 2^{19–22} representing from 34.8% to 70.8% of the studied MDR-TB cases. In the frame of our study, three TB cases belonging

to lineage 2 were epidemiologically linked; two of them were < 5 SNPs apart indicating recent transmission event.

It is well known that drug-resistance mutations often affect biological fitness leading to less competitive drug-resistant MT strains compared to drug-sensitive strains. In such isolates secondary mutations have been described in several genes (*rpoA*, *rpoB*, *rpoC*, *nusG*, *oxyR-ahpC intergenic region*) mainly in rifampicin-resistant MT strains^{14,23–28} while some other studies detected compensatory mutations also in isoniazid-resistant MT strains^{26,27}. These compensatory mutations offset the fitness cost associated with rifampicin-resistance mutations in MT, resulting in resistant isolates that are as fit as the drug-susceptible MT isolates. Concordant with the published literature, we found out that 60% (3/5) of MDR and pre-XDR MT isolates carried compensatory mutations in *rpoC* gene (Leu449Arg, Val483Gly, Ile491Val). We did not detect compensatory mutations in other drug-resistant MT isolates without rifampicin-resistance mutations. When compared to other studies, reported percentages of compensatory mutations among MDR MT strains ranged between 19.5% to 66.7%. These differences in percentages of detected compensatory mutations could be partially explained with different study designs and might even reflect different epidemiological situation in the country of the published literature.

According to our results, lineage 4 is the most prevalent MT lineage in Slovenian population being discovered in 95.32% of TB patients. Sub-lineages Haarlem (4.1.2.1) and mainly-T (4.7, 4.8, 4.8.1 and 4.9) were found in 38.42% and 30.99%, respectively. Other lineages are only sporadically discovered and mainly detected in foreigners. Lineage 4 is geographically the most widespread cause of human tuberculosis. It is found at significant frequencies across all continents^{29,30}. The predominance of lineage 4 in our setting is consistent with the reports from other European countries^{22,31,32} and other parts of the world³³.

Approximately one-fifth of TB cases in our study were likely attributable to recent transmission, whereas the remaining 81% probably resulted from reactivation of latent infection, importation of genetically distinct strains (mainly other lineages), or transmission events that were not captured within the studied population. Identification of epidemiologically linked cases supports the usefulness of WGS for detecting transmission chains. On the other hand, the large proportion of unique isolates indicates that recent transmission was not the predominant driver of TB in our population. These observations are consistent with reports from other low-incidence European countries where comparable percentage of MT isolates are clustered with WGS approach^{21,34}. Nevertheless, it should be accounted that cluster rate is affected by several factors such as sample size, sample composition, studied period, and nevertheless, quality of local and national TB control programs.

Our findings indicate that TB epidemic in our setting is characterized by the predominance of lineage 4, limited recent transmission and high prevalence of the *fabG1* -15C > T mutation among INH-resistant isolates. Detection of this mutation in both epidemiologically linked cases and genetically unrelated isolates suggests that its prevalence reflects both clonal transmission of resistant strains and recurrent

emergence under selective pressure. These results highlight the utility of WGS-based analysis as comprehensive tool for understanding the interplay between bacterial population structure, transmission and the evolution of drug resistance.

Methods:

Ethics statement

This retrospective study was approved by the National Medical Ethics Committee of the Republic of Slovenia (Ljubljana, Slovenia) with approval number 0120 – 94/2021/3 and was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki. The requirement for informed consent was waived owing to the retrospective nature of the study. The decision not to require informed consent was upheld by the National Medical Ethics Committee of the Republic of Slovenia (approval number 0120 – 94/2021/3). The study does not pose additional burdens for patients included in this study and does not reveal their personal data.

Study design and selection of *Mycobacterium tuberculosis* isolates

This retrospectively designed, single-centre study was conducted at the Laboratory for Mycobacteria (University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia). Between 2020 and 2024, all MT isolates from patients with newly diagnosed TB were routinely collected and retrospectively analysed. As the national reference laboratory, Laboratory for Mycobacteria receives and processes samples from medical centres and regional hospitals nationwide, performing diagnostic procedures at both primary and national levels. All included MT isolates had available data on phenotypic drug susceptibility testing (pDST) at least to first-line drugs. Identification of isolates was performed using Genotype MTBc according to the manufacturer's instructions (Bruker Hain Lifescience, Nehren, Germany). We retrospectively reviewed medical and laboratory records and data obtained from Laboratory for Mycobacteria and National Registry for Tuberculosis of the Republic of Slovenia (both located at University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia). The minimum metadata collected for each MT isolate included patient age, gender, date of diagnosis, and sample type.

Phenotypic drug susceptibility testing (pDST)

All MT isolates were tested for drug susceptibility to first-line drugs (isoniazid – INH: 0.1 µg/ml, rifampicin – RMP: 0.5 µg/ml (cut-off applied since September 2024 according to WHO technical report³⁵) or 1.0 µg/ml (cut-off applied before September 2024), ethambutol – EMB: 5.0 µg/ml, pyrazinamide – PZA: 100.0 µg/ml, streptomycin – SM: 1.0 µg/ml).

In case of detected resistance to at least one of first-line drugs, susceptibility testing was performed for one or several of the following antibiotics and corresponding cut-off values: moxifloxacin (MOX) 0.25 µg/ml and 1.0 µg/ml, levofloxacin (LFX) 1.0 µg/ml, amikacin (AN) 1.0 µg/ml, kanamycin (KN) 2.5 µg/ml,

linezolid (LZD) 1.0 µg/ml, clofazimin (CFZ) 1.0 µg/ml, bedaquilin (BDQ) 1.0 µg/ml, delamanid (DLM) 0.06 µg/ml, pretomanid (PTM) 0.5 µg/ml and 2.0 µg/ml, ethionamide (ETH) 5.0 µg/ml. Phenotypic drug susceptibility testing was performed using BACTEC MGIT 960 System (BD Diagnostic System, NJ, USA) according to manufacturer procedures. In case of discrepancies between pDST and WGS, pDST was repeated.

DNA isolation

DNA was isolated from pure MT cultures grown on Löwenstein-Jensen slopes. Two full loops of bacterial culture were collected into 1× TE buffer and heat inactivated for 20 minutes on 80°C. Samples were processed with cetyltrimethylammonium bromide (CTAB) method as described previously³⁶. DNA isolates were stored in 1× TE buffer at -20°C until further analysis. Isolated DNA was quantified using Qubit 1x dsDNA HS Assay Kits with Qubit 4 Fluorometer (Thermo Scientific, Willmington, USA) and purity was assessed using the NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA).

Library preparation, sequencing, data analysis and interpretation

All DNA samples were normalised to 150–250 ng in 30 µl of starting sample dilution. To identify gene variants related to drug resistance in genomic DNA, DNA libraries were prepared with Illumina DNA Prep kit and sequenced on Illumina MiSeq platform with MiSeq Reagent Micro Kit v2 according to manufacturer instructions (Illumina, San Diego, CA, USA). Before sequencing, DNA fragment length distribution was measured with the High Sensitivity DNA kit and 2100 Bioanalyzer (Agilent Technologies, USA).

FASTQ files were analysed using the PhyResSE v1.0 online tool and MTBseq pipeline v1.1.0³⁷. Paired-end reads were aligned to the reference genome H37Rv (GeneBank accession no. NC_000962.3). The variant calling was conducted with GATK to identify single nucleotide polymorphisms. The analysis was performed on the mapped MTBc reads with quality threshold of a mean coverage of at least 30x. Parameters set at MTBseq pipeline for variant call only if mutation was detected in at least 4 forward and 4 reverse reads with Phred quality score of 20 and mutation frequency ≥ 75%.

Transmission (phylogenetic) analysis:

Transmission clusters were defined by applying a threshold of 12 / 5 or fewer pairwise SNPs between sequences. Datasets were processed with MTBseq v1.1.0 pipeline to generate distance matrix. SNP-sites v2.5.1 was used to extract SNPs and to remove invariant sites from a multi-FASTA alignment. SNP-based maximum likelihood phylogenetic tree was generated with IQ tree v2.4.0 with MFP + ASC model

and branch support of 1000 bootstrap replicates. Both tools, SNP-sites and IQ tree, were used via Galaxy platform v26.0.1. The resulting phylogenetic tree was visualized using web tool Evolview v2.

Declarations

Funding

None.

Competing interests

The authors declare no competing interests.

Author Contribution

All (ES, ST, ŠK) authors designed the study. ES and ST performed library preparation, WGS data analysis and interpretation. ES wrote the first draft of the manuscript, with contributions from ST and ŠK. All authors read and approved the final manuscript.

Data Availability

Sequence data are available in SRA NCBI database under BioProject accession number PRJNA1511861 (SRA records accessible with the following link

<https://dataview.ncbi.nlm.nih.gov/object/PRJNA1511861?reviewer=77fjairfibumjhhmmad6ftart>). All other data generated or analysed during the study are included in this article.

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Tables

Table 1

Basic characteristics (age, gender) and clinical manifestation of tuberculosis (TB) for patients included in this study (N = 342)

Study cohort	N (%)	Median age (range)	Clinical manifestation of TB	N (%)
All TB patients	342 (100.00)	61.0 (1.0–96.0)	pulmonary	252 (73.61)
			extrapulmonary	90 (26.39)
Male	203 (59.24)	60.0 (1.0–93.0)	pulmonary	155 (76.23)
			extrapulmonary	48 (23.76)
Female	139 (40.76)	65.5 (1.0–96.0)	pulmonary	97 (69.78)
			extrapulmonary	42 (30.21)

Table 2

The prevalence of phenotypic drug resistance among patients with detected drug-resistant TB (N = 26)

Drug resistance	Prevalence (%)
INH	18 (69.23)
ETH	12 (46.15)
SM	9 (34.62)
RMP	6 (23.08)
PZA	6 (23.08)
EMB	5 (19.23)
FLQ	4 (15.38)
KN	3 (11.54)

Footnote: INH = isoniazid, ETH = ethionamide, RMP = rifampicin, EMB = ethambutol, SM = streptomycin, PZA = pyrazinamide, FLQ =fluoroquinolones, KN = kanamycin

Table 3

Number and percentage of Mycobacterium tuberculosis (MT) isolates according to (sub) lineage in all MT isolates and resistant MT isolates included in study

All MT isolates N = 342			Resistant MT isolates N = 26			
Lineage	Number (%)	Sub lineage	Number (%)	Number (%)	Sub lineage	Number (%)
Lineage 1	3 (0.88)	1.1.1.1	1 (0.29)	1 (3.85)		
		1.2.1.2.1	1 (0.29)		1.2.1.2.1	1 (3.85)
		1.2.2.2	1 (0.29)			
Lineage 2	10 (2.92)	2.2.1	10 (2.93)	4 (15.38)	2.2.1	4 (15.38)
Lineage 3	3 (0.88)	3	1 (0.29)	1 (3.85)		
		3.1.1	2 (0.59)		3.1.1.	1 (3.85)
Lineage 4	326 (95.32)	4	7 (2.05)	20 (76.92)	1	1 (3.85)
		4.1	6 (1.76)			
		4.1.2	21 (5.87)		4.1.2.	5 (19.23)
		4.1.2.1	131 (38.42)		4.1.2.1	6 (23.08)
		4.1.4	3 (0.88)			
		4.2.1	5 (1.47)		4.2.1	1 (3.85)
		4.2.1.1	4 (1.17)			
		4.2.2	1 (0.29)			
		4.2.2.1	3 (0.88)		4.2.2.1.	2 (7.69)
		4.3.1	3 (0.88)			
		4.3.2	1 (0.29)			
		4.3.3	4 (1.17)			
		4.3.4.2	4 (1.17)		4.3.4.2	1 (3.85)
		4.4.1	5 (1.47)			
		4.4.1.1	20 (5.87)			

All MT isolates N = 342				Resistant MT isolates N = 26		
Lineage	Number (%)	Sub lineage	Number (%)	Number (%)	Sub lineage	Number (%)
		4.5	1 (0.29)			
		4.6.2.2	1 (0.29)			
		4.7	23 (6.74)		4.7.	1 (3.85)
		4.8	79 (23.46)		4.8.	3 (11.54)
		4.8.1.	1 (0.29)			
		4.9	2 (0.59)			

Table 4

Results of phenotypic drug susceptibility testing (pDST) and corresponding mutations linked to drug resistance for resistant *M. tuberculosis* (MT) isolates (N = 26)

MT isolate identification number	Sub lineage	pDST (resistance to particular antibiotic)	Mutations linked to drug resistance
Mono resistant			
SLO5035	4.1.2.1	INH	katG Trp90*, katG Trp90Arg, ahpC – 74G > A
SLO4745	4.3.4.2	INH	katG Ser315Thr
SLO4999	4.1.2.1	PZA	no mutation detected
SLO5021	4.7	PZA	PPE35 381T > G (in case T > C the isolate would be PZA resistant ⁺)
SLO4876	4.1.2	SM	rpsL Lys88Arg
SLO4947	4.1.2.1	SM	gid Leu35fs (102delG)
SLO4958	4.8	SM	gid Ala138Pro
SLO4763	3.1.1	MOX-LFX	gyrA Asp97Tyr
SLO4832	4.1.2	KN	eis – 12G > A
Poly resistant			
SLO4779	2.2.1	INH low-ETH	fabG1 -15C > T
SLO4841	4.1.2	INH low-ETH	fabG1 -15C > T
SLO4948	4.1.2	INH low-ETH	fabG1 -15C > T
SLO4987	4.2.2.1	INH low-ETH	fabG1 -15C > T
SLO4988	4.2.2.1	INH low-ETH	fabG1 -15C > T
SLO4819	4.8	INH low-ETH	fabG1 -15C > T
SLO4860	4.8	INH low-ETH	fabG1 -15C > T
SLO4854	4.1.2	INH low-ETH	fabG1 -15C > T
SLO5037	4	INH low-ETH	inhA Ser94Ala, gyrA Val624Leu
SLO4880	4.1.2.1	SM-INH low-ETH	gid Gly34Val, fabG1 -15C > T
SLO5006	4.1.2.1	SM-INH	gid Ala205Thr, katG Ser315Thr

MT isolate identification number	Sub lineage	pDST (resistance to particular antibiotic)	Mutations linked to drug resistance
Mono resistant			
SLO4711	1.2.1.2.1	RMP-ETH	inhA – 796C > T, rpoB Gln436del (1306_1308delCAG)

Table 4
continued

MT isolate identification number	Sub lineage	pDST (resistance to particular antibiotic)	Mutations linked to drug resistance
MDR-TB			
SLO4992	4.1.2.1	INH-RMP-EMB-PZA	ahpC – 52C > A, rpoB Gln432Leu, embB Asp354Ala, pncA 147_148insGGCGATCCGTCCCGGCGTACGCC
Pre XDR-TB			
SLO5033	2.2.1	SM-INH-RMP-EMB-PZA-MOX low-LFX-ETH	rpsL Lys43Arg, katG Ser315Thr, rpoB Ser450Leu, embB Gly406Ala, pncA Ile31Ser, gyrA Ala90Val, ethA Asn114fs
SLO5047	2.2.1	SM-INH-RMP-EMB-MOX-LFX	rrs 517C > T, katG Ser315Thr, rpoB His445Tyr, embB Gly406Ala, gyrA Asp94Gly
SLO4870	2.2.1	SM-INH-RMP-EMB-PZA-MOX low-LFX-KN-ETH	rrs 517C > T, katG Ser315Thr, rpoB Leu430Pro, embA – 12C > T, embB Asp354Ala, pncA Asp136Gly, gyrA Ala90Val, eis – 10G > A, ethA – 7T > C
SLO4762	4.2.1	SM-INH-RMP-EMB-PZA-KN-ETH	rpsL Lys88Arg, fabG1 -15C > T, katG Ser315Thr, rpoB Ser450Leu, embA – 16C > T, embB Ser297Ala, pncA Pro69Arg, eis – 12C > T, ethA His281Pro
Footnote: INH = isoniazid, ETH = ethionamide, RMP = rifampicin, PZA = pyrazinamide, EMB = ethambutol, SM = streptomycin, MOX = moxifloxacin, LFX = levofloxacin, KN = kanamycin, low = low level resistance, MDR–TB = multi drug resistant tuberculosis (resistance to INH and RMP), XDR-TB = extensively resistant tuberculosis (resistance to INH, RMP, any fluoroquinolone and either bedaquiline or linezolid), pDST = phenotypic drug susceptibility testing, + according to WHO catalogue published in 2023			

Table 5

List of mutations linked to drug resistance and their prevalence in resistant *M. tuberculosis* (MT) isolates

Antibiotic	Mutation linked to drug resistance	Prevalence
INH	inhA – 796C > T	1
	katG Ser315Thr	6
	katG Trp90*	1
	katG Trp90Arg	1
	ahpC – 52C > A	1
INH, ETH	inhA Ser94Ala (low-level)	1
	fabG1 -15C > T	10
	ahpC – 74G > A	1
ETH	ethA 342delA	1
	ethA His281Pro	1
	ethA – 7T > C	1
RMP	rpoB Leu430Pro	1
	rpoB delCCAG > C	1
	rpoB His445Tyr	1
	rpoB Gln432Leu	1
	rpoB Ser450Leu	2
PZA	pncA Asp136Gly	1
	pncA 147_148insGGCGATCCGTCCCGGCGTACGCC	1
	pncA Ile31Ser	1
	pncA Pro69Arg	1
	PPE35 381T > G (v primeru T > C R)	1
EMB	embA – 12C > T	1
	embA – 16C > T	1
	embB Asp354Ala	2
	embB Gly406Ala	2
Footnote: INH = isoniazid, ETH = ethionamide, RMP = rifampicin, EMB = ethambutol, SM = streptomycin, MOX = moxifloxacin, LFX = levofloxacin, KN = kanamycin, PZA = pyrazinamide		

Antibiotic	Mutation linked to drug resistance	Prevalence
SM	embB Ser297Ala	1
	rpsL Lys88Arg	2
	rpsL Lys43Arg	1
	rrs 517C > T	2
	gid 102delG	1
	gid Ala138Pro	1
	gid Ala205Thr	1
	gid Gly34Val	1
MOX, LFX	gyrA Ala90Val	2
	gyrA Asp97Tyr	1
	gyrA Asp94Gly	1
KN	eis – 21G > A	1
	eis – 10G > A	1
	eis – 12C > T	1
Footnote: INH = isoniazid, ETH = ethionamide, RMP = rifampicin, EMB = ethambutol, SM = streptomycin, MOX = moxifloxacin, LFX = levofloxacin, KN = kanamycin, PZA = pyrazinamide		

Figures

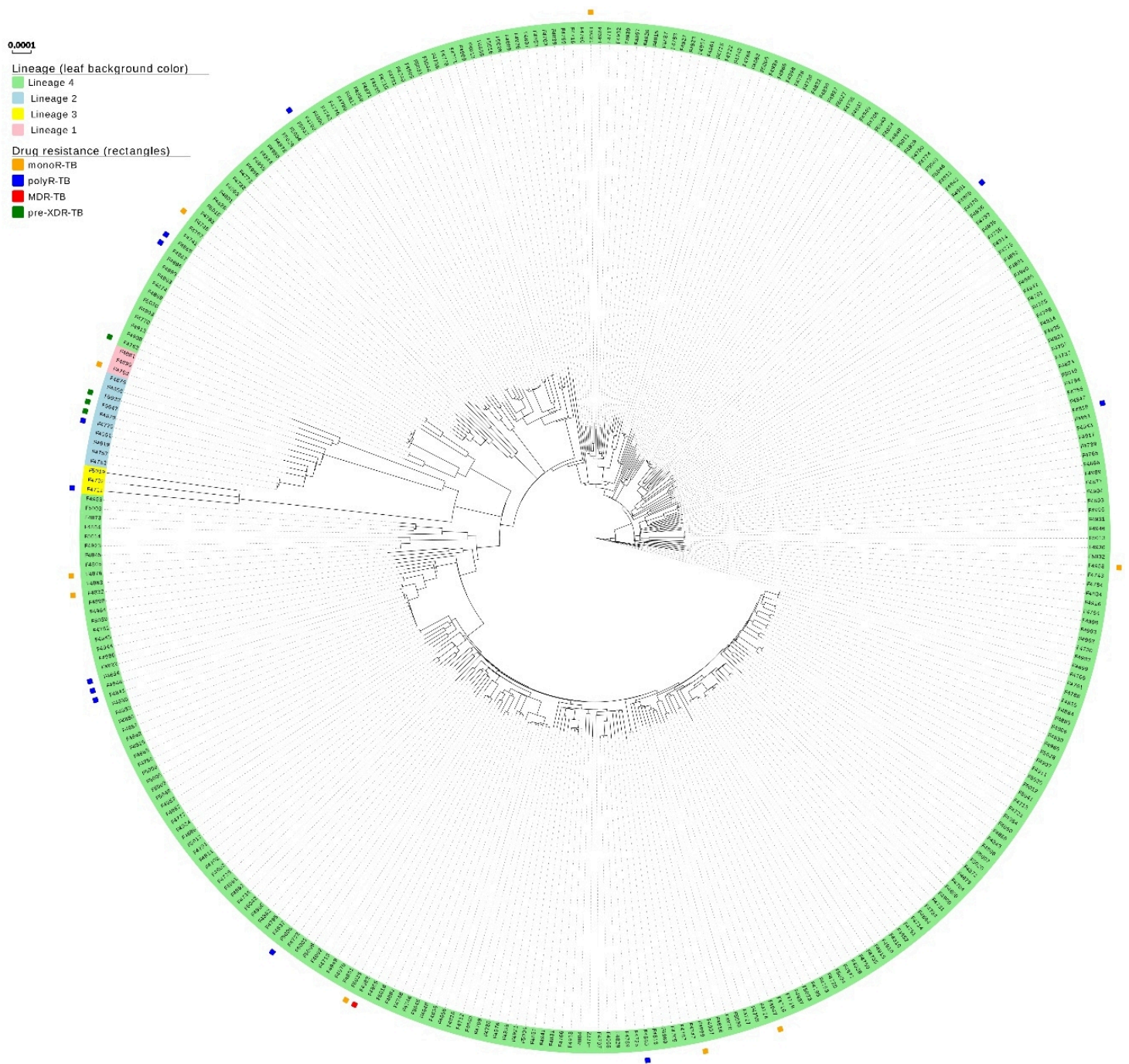


Figure 1

Maximum likelihood tree for all newly discovered MT isolates ($N = 342$) between years 2020 and 2024. SNP-based maximum likelihood phylogenetic tree was generated with IQ tree v2.4.0 with branch support of 1000 bootstrap replicates (Galaxy platform v26.0.1). The resulting phylogenetic tree was visualized using Evolveview v2. Rectangles represent corresponding drug resistance.

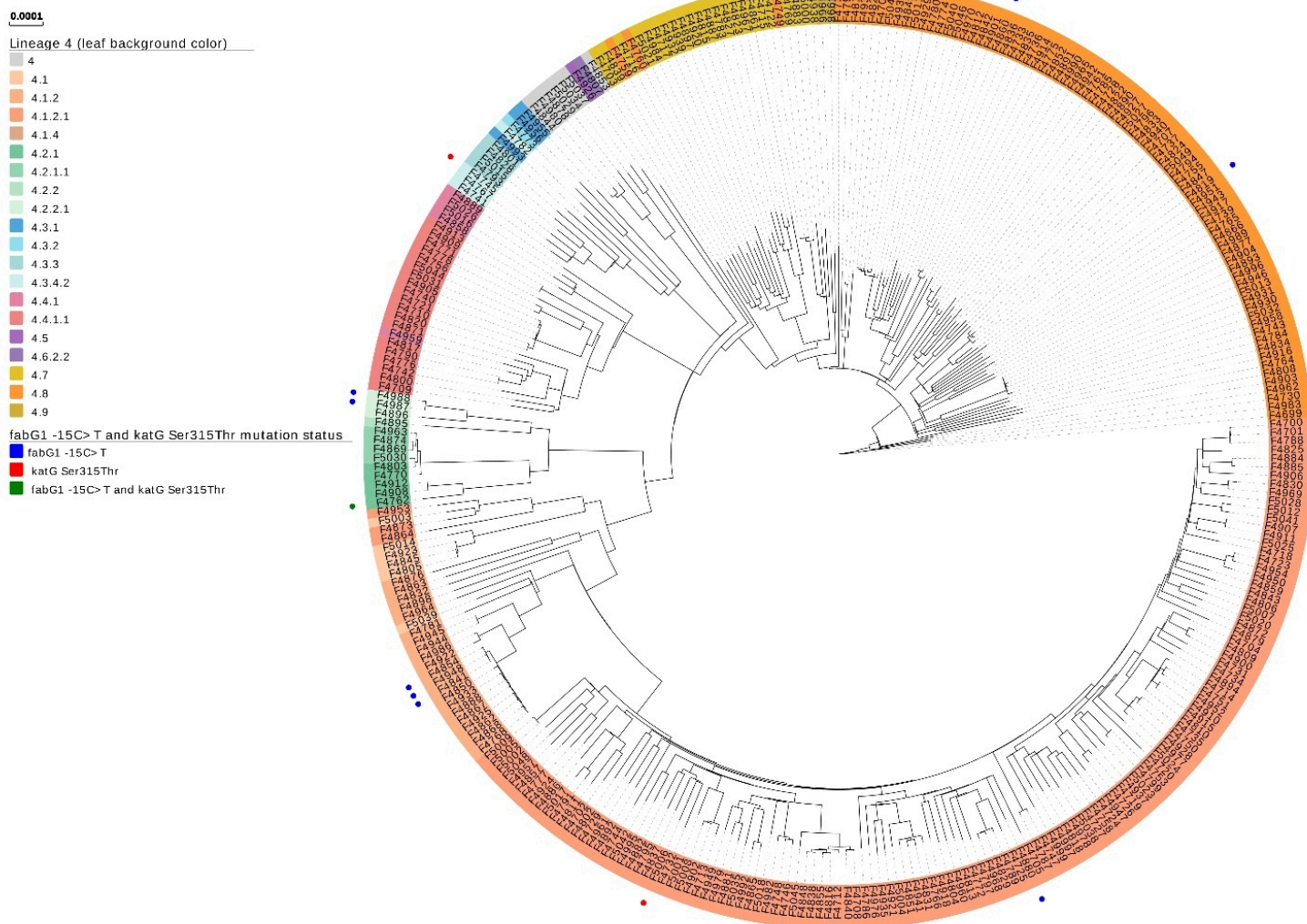


Figure 2

Maximum likelihood tree for MT isolates belonging to lineage 4 ($N = 326$) between years 2020 and 2024. SNP-based maximum likelihood phylogenetic tree was generated with IQ tree v2.4.0 with branch support of 1000 bootstrap replicates (Galaxy platform v26.0.1). The resulting phylogenetic tree was visualized using Evolview v2. Circles represent *fabG1* -15C>T and/or *katG* Ser315Thr mutation status.